

Copulation of *Neozephyrus japonicus* (Lycaenidae) under captive conditions

Michio IMAFUKU¹⁾, Takeshi OHTANI²⁾ and Tsuyoshi TAKEUCHI³⁾

¹⁾³⁾Department of Zoology, Graduate School of Science, Kyoto University,
Sakyo, Kyoto, 606-8502 Japan; e-mail: ¹⁾ima@ci.zool.kyoto-u.ac.jp

²⁾Museum of Nature and Human Activities, Hyogo, Yayoigaoka 6, Sanda, Hyogo, 669-1546 Japan;
e-mail: ²⁾ohtani@nat-museum.sanda.hyogo.jp

Abstract Observation of behavioral interactions between sexes is important for understanding of the color patterns of butterfly wings. Such observation, however, is very rare for the *Zephyrus* hairstreaks which sometimes show conspicuous sexually dimorphic coloration on their wings. Copulation of a dimorphic species *Neozephyrus japonicus* was observed in a large wire-net cage in the Museum of Nature and Human Activities, Hyogo. Out of 12 males and 10 females released, two males attempted copulation, and one successfully copulated with one female during the observation period from June 11 to 13, 1999. The copulation time was about 3 h. A large spermatophore was later confirmed in the bursa copulatrix by dissection of the female abdomen. Significance of induction of copulation in the *Zephyrus* hairstreaks is discussed.

Key words *Neozephyrus japonicus*, copulation, Theclidi, hairstreaks, *Zephyrus*.

Introduction

Subtribe Theclidi, the *Zephyrus* hairstreak, is a biologically intriguing group, because it contains a suitable number of species to consider and also because of its rich variety of morphology and modes of life (Saigusa, 1988). Furthermore, this group is interesting because of its behavioral variety; wings of some species exhibit sexually dimorphic coloration while those of others are monomorphic (Kawazoé & Wakabayashi, 1976), males of some species are perchers, whereas others are patrollers, and the activity time is species specific (Fukuda *et al.*, 1984). These characteristics may be interrelated.

Sexual dimorphism found in animal morphology and coloration may be explained in terms of intersexual or intrasexual selections (Krebs & Davies, 1981). That is, males' brilliant coloration found in some butterflies may be derived from male-male interactions or from choices of males by females.

The former is supported through field experiments by Lederhouse & Scriber (1996) and the latter may be suggested through model experiments by Silberglied & Taylor (1978) and Rutowski (1981) because females strongly responded to male colors (but see Silberglied, 1994). Compared to the species investigated, some of the *Zephyrus* hairstreaks have much more conspicuous sexual dimorphism in their wing colors, at least for human eyes.

It could be said that wing colors of some males of this group are the most conspicuous and brilliant among butterflies found in Japan. These species, however, have not yet been subjected to behavioral studies, probably because of some difficulties inherent to this group; they are all arboreal and thus difficult to observe, and also they hardly ever engage in courtship or copulation in captivity (see Discussion). Therefore, most observations on interactions between the sexes have been made accidentally in nature (ref. Fukuda *et al.*, 1984;

Kurita, 1993; Fujii, 2000), and the information on the processes preceding copulation is very limited for these species.

We had attempted to induce copulation in some species of the *Zephyrus* hairstreaks in various sized cages, but all in vain. Finally we released a number of *Neozephyrus japonicus* in a large wire-net cage in the Museum of Nature and Human Activities, Hyogo, and succeeded in observing two instances of copulation attempts and one of copulation. Here we report the results of this observation.

Materials and methods

Larvae of *Neozephyrus japonicus* were collected from the food plant of *Alnus* spp. in Kyoto and Ibaraki Cities between May 5 and 15. They were reared separately in plastic cases (10 cm dia., 4.5 cm ht) with leaves of the food plants. Butterflies emerged from May 20 to June 1, and were maintained in the same case with a cotton ball soaked with sugar solution as adult food. To avoid exhaustion, butterflies were kept in a refrigerator at about 10°C for 1 week until two days before observation.

Observations were made in the wire-net cage (Fig. 1) built at the Museum of Nature and Human Activities, Hyogo, located in Sanda City, Hyogo Prefecture from June 11 to 13. The cage was rectangular, 11.6 m long and 7.7 m wide with the longer side facing east. The ceiling made of wire net was trapezoid, with the highest point being 4.0 m. There was a small room (2.5×2.9 m, 3.9 m ht) at the northwest corner. The floor of the cage was made of natural earth with free propagation of common plants such as *Erigeron annuus* and *Artemisia princeps* (Compositae). There were two trees in the cage; a *Clerodendron trichotomum* (Verbenaceae) tree of about 3 m height near the north wall and a *Phellodendron amurense* (Rutaceae) tree of about the same height at the southeast corner.



Fig 1. The inside of the observation cage with a *Clerodendron trichotomum* tree (center) on the top of which copulation was observed.

Twenty-two butterflies (12 males and 10 females) were released in the cage at 15 : 00 on June 11. Before release, they were individually marked on the margin of the ventral wing surface with a pink colored felt pen (Marker PX-20, Mitsubishi). Temperature was measured in the shadow of the *Clerodendron* tree at height of 1 m, and the light intensity was measured in the cage with a Pocket Lux (Lichtmeßtechnik, Berlin), facing upward. For observations at higher places, a stepladder was used.

Results

1. Observations on June 11

When released at 15 : 00 on June 11, some butterflies flew to the net wall, and others flew to the *Clerodendron* tree or alighted on the grass. No apparent activities were observed on that day, except for some individuals bathing with the wings opened or occasionally flying spontaneously.

2. Observations on June 12

1) Inactivity till 16 : 00

When observation was started at 7 : 00 (21.4°C, 13,800 lux) on the next day (June 12, clear), half of the butterflies were bathing on the net walls, on the tree, or on the grass. In daytime, the butterflies were relatively inactive, and rarely flew from one place to another. When the sunlight became stronger and the temperature rose, the number of butterflies on the *Clerodendron* tree increased, with no clear difference between males and females (Fig. 2). No butterflies were found on the other *Phellodendron amurense* tree. At 12 : 00, the temperature was 27.9°C and the light intensity was 51,900 lux. At 14 : 00 (28.2°C, 34,000 lux), many butterflies were found staying in the shade of the *Clerodendron* tree, some perching on the underside of the leaves.

2) Behavior before spiral flight

Around 16 : 00 (26.2°C, 15,800 lux), some butterflies initiated bathing and occasional flew around. At 16 : 45 two individuals flew in an entangling manner, but not in a spiral flight typical for the *Zephyrus* hairstreaks (traditionally "manjidomoe" flight, in Japan, Fukuda *et al.*, 1984, or co-rotating flight, Sibatani, 1998). At 16 : 50 many butterflies flew around within the cage. Such flights did not last for long. When one butterfly took off, many others entered flight as if enticed. Such behavior continued. At 17 : 30, one spiral flight occurred.

3) Copulation of ♀ 8 and ♂ 6

At 17 : 50, one butterfly chased another in a large circular movement of about 5 m radius, and then the two almost simultaneously perched on top of the *Clerodendron* tree. Their positions were on two separate leaves 15 cm apart. Then the two simultaneously took off and again simultaneously alighted, this time, on the same leaf. When observed at close range, one individual (♂ 6) very slowly walked toward the left side of the other (♀ 8) that was perched 1 cm away. At that time, the pair of antennae of the male were widely opened, each at 90° or more from the front (*cf.* Fig. 3). After a slow approach, the male gradually changed its position to arrange itself parallel to the female, both of them facing in the same direction. It took about one minute to assume the parallel position. In this position, the

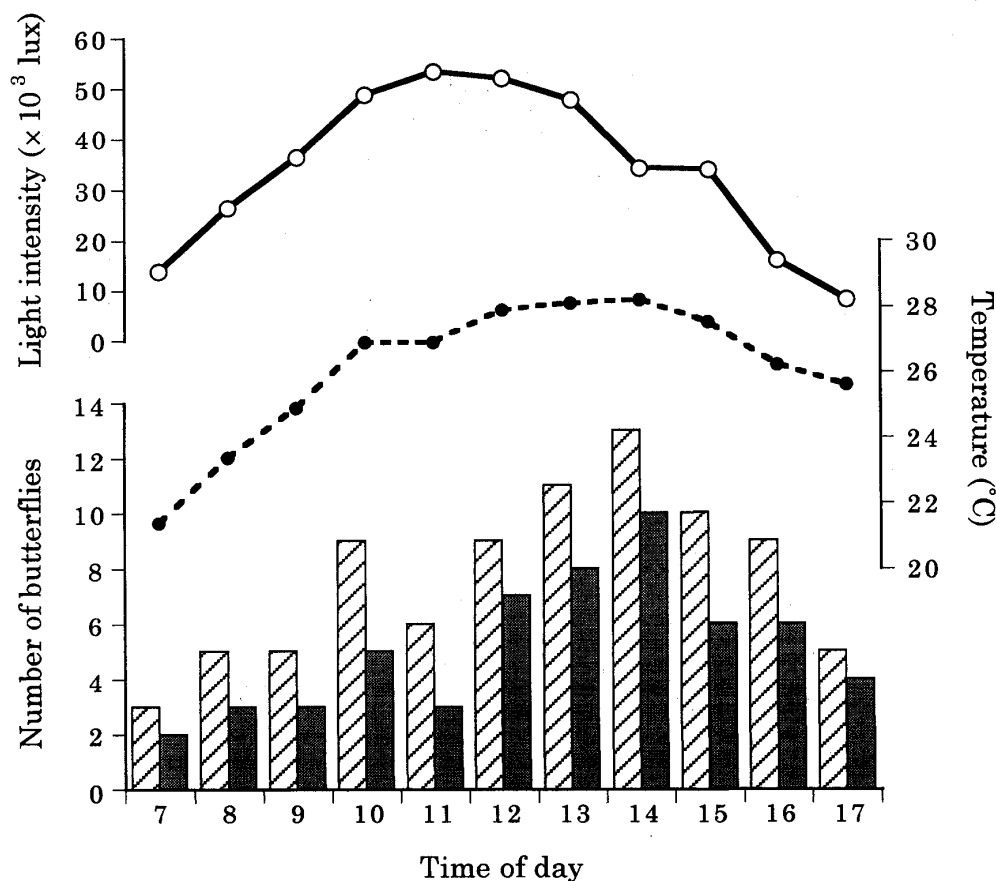


Fig 2. Diurnal change of the number of butterflies on the *Clerodendron* tree. The striped and solid sections indicate females and males, respectively. Temperature is shown with a dashed line, and light intensity with a solid line.

antennae of the male had returned to their normal position, pointing obliquely forward.

Then, the male curved its abdomen to the right and neared its abdominal tip to that of the female. But it did not succeed in connecting. When the female walked slightly away, the male again approached (17:53'17'') in the same way as previously, and then assumed the parallel position (53'54'') again. After connecting the genitalia, the male, but not the female, changed its direction to attain the normal end-to-end copulation positions (54'26''). However, this connection seemed imperfect, and the male repeated the process from assuming the parallel position (56'51'') to the copulation position (57'46''). Then they remained still till 18:03. Then, they separated and assumed the parallel arrangement once more (18:06). This time, the female sidled up nearer to the male, but nothing happened thereafter for several minutes.

After a short flight (18:15), the male made two unsuccessful copulation attempts with the same female (in one of them, the male attempted copulation from the right side of the female, a position opposite to the other attempts) and finally succeeded (18:26) after a normal approach (Fig. 3). To address the copulation time, the two were observed intermittently. They were found in copula (*cf.* Fig. 4) at 21:00 (21.3°C), and they had separated by 22:00. After separation, the two were in almost the same juxtaposition as before copulation (Fig. 5). The copulation time was 2.5 to 3.5 h. The female (φ 8) was captured and was kept separately for later examination.

3. Observations on June 13

The next day (June 13) varied from partly cloudy to cloudy. The behavior observed on that day was almost identical to that seen on the previous day from morning to noon; they were relatively inactive around noon. At 12:37 (25.0°C, 19,800 lux), ♂ 16 performed patrol flights; it flew over the *Clerodendron* tree and returned nearly to the same position on the tree. At 16:32 (24.0°C, 9,750 lux at 16:00), ♂ 10 was found attempting copulation with ♀ 14 on top of the *Clerodendron* tree (Fig. 6), but they were accidentally disturbed by the observer, and the observation was terminated.

4. Dissection of ♀ 8

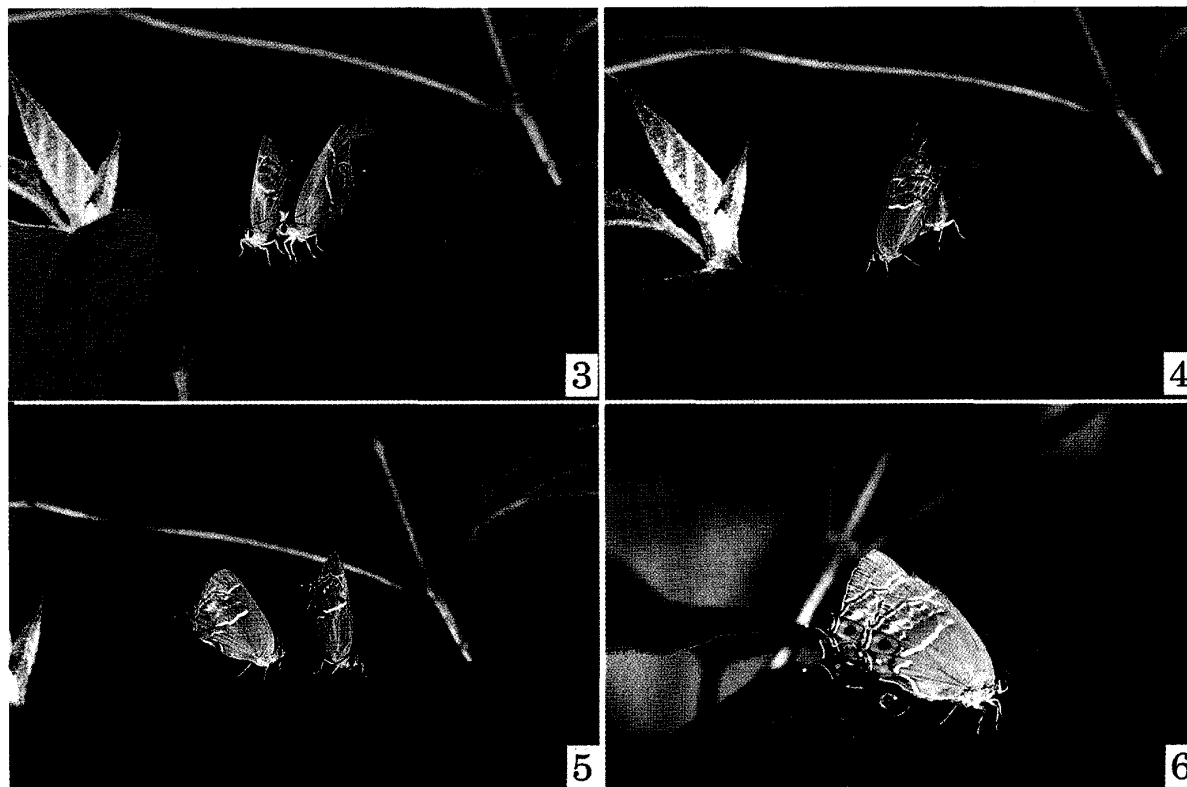
To confirm the effectiveness of the copulation observed between ♀ 8 and ♂ 6, the former was kept with the food plant in a plastic case (20×13, 13 cm ht) for one week after the copulation. But no oviposition was attained, and the female became gradually exhausted. The female was dissected for the presence of spermatophores on July 2 as suggested by Dr Yoshiaki Obara of the Tokyo University of Agriculture and Technology. One large white spermatophore was found in the bursa copulatrix, together with some greenish eggs in the ovarioles.

Discussion

A male of *Neozephyrus japonicus* successfully copulated in the large net-cage at the Museum of Nature and Human Activities, and the copulation was confirmed to be effective, because the copulated female possessed a large spermatophore in the abdomen. Here we discuss copulation of the *Zephyrus* hairstreaks in captivity and some aspects concerning courtship and copulation of *N. japonicus*.

Copulation in captivity

Successful induction of copulation in captivity is rare for the *Zephyrus* hairstreaks. Taketsuka (1973) could not induce copulation of *Artopoetes pryri* in plastic bags, but he (1973, 1976) could do so by releasing some receptive females in nature. We did not succeed in inducing copulation in a medium-sized cage (45×45, 60 cm ht) placed outdoors for *Neozephyrus japonicus*, *Sibatanozephyrus fujisanus*, *Wagimo signata* and *Araragi enthea*, or in a rather wide mosquito net (360×360, 180 cm ht) set indoors or in the field for *Chrysozephyrus ataxus*, *Neozephyrus japonicus* and *Favonius saphirinus*. Contrary to these trials, a few species are known to copulate in captivity. Kudo (1993) observed twice the copulation of the same pair of *Japanica onoi* in a cage. According to Odagiri (*pers. comm.*), copulation can be induced in caged conditions for *Japanica lutea* and *Artopoetes pryri*. All these successful species are sexually monomorphic for wing coloration. Our observation of copulation in captive *Neozephyrus japonicus* seems to be the first record among dimorphic species of the *Zephyrus* hairstreaks. On the other hand, behavior from courtship to copulation has been occasionally witnessed in nature for several species: *Neozephyrus japonicus* (*cf.* Fujii, 2000), *Sibatanozephyrus fujisanus* (*cf.* Kurita, 1993), *Chrysozephyrus brilliantinus* (*cf.* Nakai, 1979; Kurita, 1993), *C. hisamatsusanus* (*cf.* Nishida, 1994), *C. ataxus*, *C. smaragdinus*, *Antigius attilia*, *Wagimo signata* (*cf.* Fukuda *et al.*, 1984), *Shirozua jonasi* (*cf.* Sato & Yamauchi, 1982; Fujii, 1989; Odagiri, 1993), *Coreana raphaelis* (*cf.* Hirano, 1977; Kusakari, 1992) and *Artopoetes pryri* (*cf.* Taketsuka, 1973, 1976). Why are they reluctant



Figs 3-6. Copulation behavior of *Neozephyrus japonicus*. 3. ♂6 that approaches ♀8, widely opening a pair of antennae (18:22 June 12). 4. In copulation (20:00). 5. After copulation (22:00). 6. Copulation attempt with ♀14 by ♂10 (16:32 June 13).

to copulate in confinement? Males seem to be responsible, because they rarely show behavior that leads to copulation, such as approaching the female or curving the abdomen towards a female, even when they were artificially brought into approximation to the female (Imafuku, *pers. obs.*). Although it is possible that the females may not activate the males by, for example, sending any signs about their receptivity through certain behavior or pheromone, a major cause of reluctance to copulate seems to be attributable to the male condition, because males are known to be usually active in sexual behavior (Krebs & Davies, 1981; Ohtani & Yamamoto, 1985) and are observed in nature attempting to force copulation (Ohtani, 1988; Rutowski, 1997) or to court even homosexual individuals or heterospecific females, *e. g.*, *Favonius ultramarinus* males and a conspecific male (Kurita, 1993) or an *F. yuasai* female (Fujii, 2000). If our males were sexually active, more copulation attempts or courtship would have been observed in the cage. Thus, control of the condition of males seems to be important for copulation induction in the *Zephyrus* hairstreaks. In the large cage, some *N. japonicus* males exhibited patrol flight and spiral flight, and finally performed copulation. These facts suggest that generous space is needed for copulation of dimorphic species. It is not conclusive, however, because copulation was observed only once.

In the cage, *N. japonicus* males did not easily succeed in copulation, taking some time for copulation attempts prior to being successful. This is in contrast with observations made in nature. Almost all of the copulations observed in nature for the *Zephyrus* hairstreaks are established immediately, or at most within 10 s; *Chrysozephyrus brilliantinus* (*cf.* Nakai, 1979; Kurita, 1993), *C. hisamatsusanus* (*cf.* Nishida, 1994), *C. ataxus*, *Wagimo signata* (*ref.* Fukuda *et al.*, 1984), *Artopoetes pryori* (*cf.* Taketuka, 1973), *Coreana raphaelis* (*cf.* Hirano,

1977) and *Shirozua jonasi* (cf. Sato & Yamauchi, 1982; Fujii, 1989; Odagiri, 1993). In *Colias*, copulation took 5 s to establish (Silberglied & Taylor, 1978). In *Pieris* it was within 10 s (Ohtani, *pers. obs.*), and the male behavior was described in detail (Ohtani, 1985, 1988). Unsuccessful attempts observed in our butterflies may be attributed to the artificial caged condition or preservation of the butterflies in the refrigerator before observation.

In confinement, there may be some other unsuitable or harmful factors. In our brief observations, the pale grass blue *Zizeeria maha* males easily and quickly copulated with virgin females released in nature, but it took much longer to copulate in a small plastic case (10 cm dia., 4.5 cm ht). In one case, a male on the wall attempted copulation with a female alighted on the ceiling. The corner between the wall and the ceiling seemed to interfere with copulation. In another case, a *Zizeeria maha* male curved its abdomen and extended its genitalia to within 3 cm from the receptive female. This seemed to be induced by some odor emitted from the female, as female odor was shown to sexually activate males (Wago, 1978). Though observations of behavior on captive animals frequently provide detailed information, we should be careful with such potential effects.

Courtship behavior and copulation

Behavior shown by the caged *N. japonicus* butterflies is thought to be natural in some aspects. They were relatively inactive during the daytime and became active in early evening after 16:00. They did patrol flights on the top of the tree. These activities were usually observed in nature (Fukuda *et al.*, 1984). Further, a spiral flight was also observed in the cage, as known in the field (Kurita, 1993; Imafuku, *pers. obs.*). There is no detailed report on the copulation of this species in nature (Fukuda *et al.*, 1984). The results of the present observation suggest that copulation of this species in nature occurs near tree-tops at dusk.

Prior to copulation, the two butterflies (♀ 8 & ♂ 6) performed some short flights. In those flights, they took off and alighted almost simultaneously. Simultaneous take-off by two butterflies was also observed in our previous observations made in a cage or in a mosquito net for *Chrysozephyrus ataxus* and *Sibatanozephyrus fujisanus*. Though such flight by females seems to be different from so-called solicitation flight that includes approach and chase by females (Rutowski, 1980), it may be considered to be a part of precopulatory behavior.

In courtship or in a slow approach, the *N. japonicus* male opened its antennae widely. The same behavior was also observed in *Shirozua jonasi*; a pair of antennae of the male were pointed, respectively, toward the head and the tip of the abdomen of the female (Odagiri, 1993). For this phenomenon, there seems to be no special meaning except for removal of the antennae from the female side which is approached, because the male usually curved only the antenna on the female side to any extent when it approached in at an oblique angle. Wide opening or drawing back of the antennae at approach may be a general phenomenon in butterfly courtship.

From our observation, the copulation time of *N. japonicus* was found to be 2.5–3.5 h. This time is not widely different from that known for other species; 3–4.5 h in *Coreana raphaelis* (cf. Kusakari, 1992), more than 2.5 h in *Shirozua jonasi* (cf. Fujii, 1989) and more than 3.2 h in *Favonius ultramarinus* (cf. Kurita, 1993). In *Colias*, it was approximately 1 h (Silberglied & Taylor, 1978). In *Pieris*, it was 0.34–7.43 h (Ohtani & Yamamoto, 1985).

One of the purposes of our observation on the *Zephyrus* hairstreaks is to obtain some hint

on the cause of sexually dimorphic coloration seen in this group; brilliant male color may be derived from female choice. In courtship, the *N. japonicus* male did not open the wings, or display his brilliant wing color to the courted female. Wing opening at courtship has been known in some other lycaenid species; *Zizeeria maha* (cf. Wago, 1976) and *Lycaena phlaeas daimio* (cf. Suzuki, 1976). No such behavior was reported for the *Zephyrus* hairstreaks, except for *Shirozua jonasi* males (sexually monomorphic species) which opened to about 30° at courtship (Fujii, 1989).

Though *N. japonicus* males seem not to open their wings at courtship, the females of this species may have a chance to perceive male wing colors when they perform a short flight maneuver prior to copulation. Generally, male butterflies fly to and land in the vicinity of the female which they court, which should provide a chance for the female to see the dorsal wing surface of the approaching male. Some flight prior to copulation is known for *Chrysozephyrus brilliantinus* (cf. Nakai, 1979), *C. ataxus*, *Antigius attilia*, *Wagimo signata* (ref. Fukuda *et al.*, 1984) and *Shirozua jonasi* (cf. Sato & Yamauchi, 1982). In *Colias*, the female-located male lowers its wing on flight near the female, and thus there is "no doubt that the upper surfaces of approaching males are visible to females" (Silberglied & Taylor, 1978: 217). Thus, it is possible that the *N. japonicus* female perceives the wing color of the flying male.

But *N. japonicus* butterflies are known to fly in almost dark conditions, so male coloration may be difficult to see for females. Males of this species, however, emit ultraviolet light twice as strong as visible green light (Imafuku, unpublished), and ultraviolet sensation is also confirmed in some species of this group (Arikawa & Imafuku, unpublished). At present there is no evidence that supports perception of male colors by females at courtship, or any evidence that denies it. Further observations are needed. We believe that successful copulation in captivity shown in the present study will contribute to the solution of the above problem.

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摘 要

ミドリシジミのケージ内での交尾 (今福道夫・大谷 剛・竹内 剛)

蝶の翅の色彩パターンの理解のためには、雌雄間での行動的相互交渉の観察が重要である。しかし、翅の色彩にしばしば著しい性的二型を示すゼフィルス類については、そのような観察は非常に少ない。そこで、性的二型の種であるミドリシジミ *Neozephyrus japonicus* の行動を兵庫県三田市の「人と自然

の博物館」にある縦 12 m, 横 8 m, 高さ 4 m の金網のケージのなかで 1999 年 6 月 11 日から 13 日にかけて観察した。ペイントマーカーで個体識別した♂ 12 頭と♀ 10 頭を放したところ, 2 回の求愛行動と 1 回の交尾が観察された。交尾に先立ち, ♂は♀に側面から触角を広げて接近し, 次第に平行に並ぶように向きをかえて, 腹端を♀の腹端に近づけた。交尾は容易に成功せず, しばしば上記の行動を繰り返したり, 短い飛翔を行ったが, 夕方の 6 時 26 分に交尾に成功した。交尾時間は約 3 時間であった。交尾後♀を食草と共に保ったが, 産卵は確認できなかった。後にこの♀の腹部を解剖したところ, 大きな精胞が 1 つ見つかった。捕獲下でのゼフィルス類の交尾の誘導の意味について議論した。

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